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Effect of dilute sulfuric acid pretreatment on the physicochemical properties and enzymatic hydrolysis of coffee cut-stems

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ABSTRACT

Coffee-cut stems are a potential fermentable sugars source, which can be upgraded in different value-added products and energy vectors. Nevertheless, there are few reports focused on the acid pretreatment and saccharification processes. Thus, this paper evaluates the effect of the acid pretreatment and saccharification conditions of coffee cut-stems to find the highest sugar yield. Thereafter, the influence of the residence time in the acid pretreatment and the β -glucosidase supplementation in the saccharification process were analyzed. The combined severity factor and crystallinity index were used as metrics to evaluate both processes. In all assays, an increase in the crystallinity index was observed. Furthermore, a nonlinear trend of the combined severity factor respect to the residence time in the acid pretreatment was evidenced. The highest sugar yield was 66.75% with a combined severity factor of 1.84. The better saccharification process was achieved at combined severity factor of 2.01 with a digestibility of 43%. The addition of β-glucosidase in the enzymatic hydrolysis allows increasing the value to 69.07%. Hence, low temperatures, acid concentrations, and the β-glucosidase supplementation allows obtaining a high sugar yield from coffee cut stems.

1. Introduction

The use of lignocellulosic biomass as feedstock is an increasing tendency in developing and developed countries as an alternative to decrease the excessive use of non-renewable resources [1]. Lignocellulosic biomass has been studied to produce different value-added products and energy vectors [2]. Moreover, this type of biomass has several advantages in comparison with other biomass sources (e.g., abundance, non-food competition, and low cost) [3]. Therefore, the valorization of lignocellulosic residues represents an opportunity to increase the incomes of a crop. In this way, the lignocellulosic residues from the coffee crop and processing (i.e., coffee-cut stems, spent coffee grounds, pulp, husk, and coffee silverskin) have a high potential to be upgraded in different marketable products. Indeed, a special interest has been focused on the potential use of coffee-cut stems (CCS) as feedstock in different biorefinery systems in the Colombian context [4].

CCS are considered as a potential feedstock owing to the high content of natural biopolymers such as glucan, xylan, mannan, arabinan, and galactan, which can be converted into soluble C_6 and C_5 sugars [5]. Moreover, CCS are a potential feedstock due to the high production rate (i.e., 0.6 kg per kg of coffee cherry processed). In fact, the production rate of CCS was 60 ton/h in 2017 [6]. However, the research focused on assessing the feasibility of CCS as feedstock to be upgraded though biotechnological processes has been incipient. For instance, Quintero et al. [7], reported a technical and economic study of the use of CCS as feedstocks to produce bioethanol. These authors reported that the total production cost using CCS is higher than the bioethanol production costs using other lignocellulosic residues. This result is attributed to the raw materials costs assumed in the economic evaluation of the process. In addition, the high bioethanol production costs can be attributed

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Abbreviations

Abbreviations

CCS	Coffee cut-stems
CrI	Crystallinity Index
CSF	Combined Severity Factor
ED	Enzymatic Digestibility
GHMF	Glucose consumed in the synthesis of HMF
HMF	Hydroxymethylfurfural
HPLC	High Performance Liquid Chromatography
NREL	National Renewable Energy Laboratory
PHB	Polyhydroxybutyrate
SL	Solid Loading
SY	Solid Yield
TSR	Total Sugars Released
XF	Xylose consumed in the synthesis of Furfural
XRD	X-ray Diffraction

Units

°C	Celsius degrees
%w/w	% weight per weight
μm	Micrometer
cm	Centimeter
db	Dry basis
FPU	Filter Paper Unit
g	Gram
h	Hour
Kg	Kilogram
L	Litter
min	Minute
mL	Milliliter
mm	Millimeter
N	Normality
rpm	Revolutions Per Minute
t	Residence time

to the glucose yield calculated by the authors. In fact, the pretreatment and saccharification stages were calculated using a kinetic model developed for corn stover [8]. Therefore, the unselective use of a kinetic model to describe the behavior of different lignocellulosic materials in the pretreatment and saccharification stages is not the best approximation to evaluate the potential use of lignocellulosic biomass. Aristizábal et al. [14], corroborated this-statement through the experimental work to obtain xylose and glucose yields derived from the dilute acid pretreatment and enzymatic hydrolysis of sugarcane bagasse, rice husk, fique bagasse, and CCS. Nevertheless, the main concern identified in the saccharification process is the overall glucose yield obtained for CCS (i.e., 19%), which is lower than the yields reported for sugarcane (i.e., 33%) and fique bagasse (i.e., 40%). These results and the glucose yields reported for other lignocellulosic residues are obtained due to the conditions handled during the pretreatment stage (i.e., 115 °C, 30 min and 5 h). In fact, the dilute acid pretreatment stage must be performed considering the structure of the raw material, which allows improving the yields obtained in this process as well as the selection of the best operation conditions according to the type of biomass to be treated. In the CCS case, this raw material has a harder and more rigid structure than sugarcane and fique bagasse, which involves the use of more severe conditions. This statement is based on the plant taxonomy. Thus, the results obtained from a pretreatment stage depends strongly on the raw material and the dilute acid pretreatment conditions selected [9].

The operating conditions of the dilute acid pretreatment can change according to the raw material to be pretreated and the process requirements. The main variables to be considered in this pretreatment are the temperature, acid concentration, residence time and solids loading. In this sense, the temperature of the process can be in the range 100 °C–200 °C. Moreover, the acid concentration can vary between 0.5% and 2.5% [18]. On the other hand, the solids loading and reaction time can be in the range 20–100 min, and 10%–15%, respectively [4]. Nevertheless, the temperature and residence time have a great influence on the generation of inhibitory compounds (e.g., aldehydes and phenolic acids), which should be considered before performing the pretreatment of a lignocellulosic feedstock [21]. Therefore, the study of this pretreatment can be handled from two perspectives. The first perspective is the optimization to reach a maximum yield of glucose. The second option is to describe the mechanism of the reactions through a kinetic model [22]. Even though, both perspectives should be researched for specific lignocellulosic materials due to the physicochemical properties vary significantly from one raw material to another. Then, the research at moderate conditions could help to identify the feasibility of new lignocellulosic materials in the biotechnological process.

CCS are a potential source of sugars after a pretreatment and saccharification stages. Nevertheless, the low enzymatic hydrolysis yields, as well as the low xylose yields reported in the literature, encourage to research the best way to pretreat this lignocellulosic residue. Therefore, the aim of this paper is to assay the effect of the dilute acid pretreatment operating conditions as well as the enzymatic hydrolysis of CCS to increase the total glucose yield. For this, a temperature of 120 °C and five residence times were assessed in the dilute sulfuric acid pretreatment. Then, the effect of the β-glucosidase supplementation in the enzymatic hydrolysis was the main variable analyzed in saccharification process. In this way, the influence of the pretreatment on the physicochemical properties of CCS, the composition of hydrolysates and the conversion of glucose was analyzed through the combined severity factor (CSF) parameter. Finally, the mass balances of both processes (i.e., dilute acid pretreatment and enzymatic hydrolysis) are presented to elucidate the potential use of this raw material to be upgraded through biotechnological processes.

2. Methodology

2.1. Raw material and chemicals

Coffee crop is one of the most important and representative crops in Colombia. The CCS are the waste generated from the renewal of this cultivation [10]. The CCS samples were supplied by a local farmer in Manizales (5° 4′ 1″ N 75° 31′ 1″ W). This city is situated in Caldas, which belong to the coffee growing areas located in Colombia. This raw material was washed using tap water until removing soil particles. CCS must be conditioned for both chemical composition analysis and dilute acid pretreatment. For this, the total amount of raw material was divided into two batches. CCS used in the acid pretreatment were dried at 45 °C, while CCS used in the chemical composition analysis were dried at 105 °C. Then, the dried solids were cut into small chips of 2.5 cm and milled in a two stage process using a laboratory scale knife mill (Thomas-Wiley mill). The milled CCS were sieved using ASTM standard

sieves between 20 and 80 mesh. The retained fraction in the ASTM 40 sieve was stored at 20 °C until use. Finally, the CCS were characterized according to the standard procedures developed by the National Renewable Energy Laboratory (NREL) [11].

The dilute sulfuric acid solution for the pretreatment step was prepared with a sulfuric acid at 95 %w/w. This acid solution was standardized using a standardized solution of sodium hydroxide 0.10 N. The chemical reagents were purchased to Merck Millipore. The enzymes used in the saccharification stage were given by the Novozymes enterprise. On the other hand, the DNS method chemicals were purchased to Panreac. Finally, the chemicals used in section 2.4.1 were supplied by the Centre of Biological Engineering of the University of Minho (Braga, Portugal).

2.2. Dilute sulfuric acid pretreatment

2.2.1. Selected conditions

The process conditions of the dilute sulfuric acid pretreatment such as acid concentration, solids loading, temperature, and residence time were selected considering the principle of this pretreatment in terms of the combined severity factor (CSF). Previous results in the open literature for the CCS and similar lignocellulosic biomass results (i.e. hardwoods such as Aspen, Poplar, Eucalyptus, Maple, and Olive tree) also were considered to propose the pretreatment conditions [10,12–17]. Therefore, this process was performed in batch mode with a solid to liquid ratio of 1:10 (g CCS, db/g) using a sulfuric acid solution of 2 %w/w and a temperature of 120 °C. Moreover, the pH value of the dilute sulfuric acid solution was 0.83. Different residence times were applied in the pretreatment process. The CSF was used as parameter to decide the best set of variables. Yang et al. [13], reports the use longer residence times or higher temperatures when the concentration of the sulfuric acid solution is low. Therefore, the residence time in this study was varied from 20 min to 180 min. Moreover, the residence time was changed to identify the effect on the saccharification process and the sugars released of the hemicellulose fraction. The CSF tested was calculated with equation (1) and presented in Table 1.

$$CSF = log\left(e^{\frac{T(^{\circ}C) - 100}{14.75}} \cdot t(min)\right) - pH \quad (1)$$

2.2.2. Laboratory procedure

A laboratory-scale batch reactor configuration conformed by glass flasks and an autoclave equipment were used to achieved the pretreatment operating conditions. Indeed, 8 g of milled and dried CCS were added into 500 ml SCHOTT flasks. Then, the dilute acid solution was added until complete the solid to liquid ratio assuming a complete humectation of the raw material. Finally, the flasks were autoclaved at 120 °C and 1 atmg using a laboratory scale autoclave (MLS-3751, Sanyo). Each assay was performed in duplicate. Then, the liquid fraction was removed by filtration. This fraction was placed on falcon tubes to withdraw 2 ml of sample. Then, this liquid sample was filtered using filters (0.22 μm) and kept at −20 °C until sugar analysis. Instead, the solid fraction was washed because of inhibitors presence and the low pH achieved using distilled water until reach a pH of 4.8. This pH value was set to perform the enzymatic hydrolysis. The required water to reach the desired pH value was 2.5 L at room temperature, which suggests a high water footprint of the dilute acid pretreatment [18]. Then, the solid fraction was divided into two fractions. The first solid fraction was oven-dried at 60 °C to perform the calculation of the solid yield (SY) as well as to carry out the composition analysis of the pretreated CCS. The SY was defined as the ratio between the dry matter of CCS recovered from the pretreatment to the dry matter of raw material employed in the process. The second solid fraction was used in a saccharification process.

2.3. Saccharification process (enzymatic hydrolysis)

The saccharification process was accomplished using Cellic CTec2 as enzyme cocktail provided by Novozymes (Denmark). The cellulose activity was measured following the NREL method [19]. In fact, the activity of the Cellic CTec2 was estimated in 123 FPU/mL, which is in agreement to the enzyme activity reported by Zhao et al., [30]. Then, the enzymatic hydrolysis of the pretreated CCS was performed. This process was carried out inside an incubator with a fixed temperature, orbital agitation and time (i.e., 50 °C, 150 rpm, and 72 h). The total volume of the saccharification process was 50 mL using a 0.05 N sodium citrate buffer solution. The solid and enzyme loadings of the process were 5% w/v and 20 FPU/g dry substrate, respectively. The samples analysis was performed based on 1 mL, and applying the DNS method [20]. A β-glucosidase supplementation was used to evaluate the effect of this enzyme in the total glucose yield of the process. For this, the β-glucosidase used had a protein concentration of 19.9 mg/mL and activity of 17.93 UIpNPG/mg protein in a ratio of 0.94 FPU/UIpNPG [21]. The β-glucosidase supplementation was done after to identify the best sugars yields using only Cellict CTec2. The results of this process were estimated using Equations (2) and (3).

$$\%\ \text{Enzymatic Digestibility(ED)} = \frac{C_{Glucose}}{1.11\ Glucan_{Substrate}\ SL} \times 100 \quad (2)$$

$$\%\ \text{Total glucose yield} = \frac{C_{Glucose}}{1.11\ Glucan_{CCS}\ SL}\ x\ 100 \quad (3)$$

where $C_{Glucose}$ is glucose concentration (g/L), $Glucan_{Substrate}$ is the amount of cellulose (i.e., glucan) in the treated CCS, $Glucan_{CCS}$ is the cellulose content in the feedstock, SL refers to the solid loading for the enzymatic hydrolysis (50 g/L), and 1.111 is the conversion factor of glucan to glucose based on a stoichiometric equation [22].

2.4. Analytical methods

2.4.1. Chemical characterization of treated CCS and hydrolysates analysis

The characterization pretreated CCS was completed using a two-step acid hydrolysis. The extractives content of the raw material was quantified applying a soxhlet extraction using water and ethanol as solvents [23]. The two-step acid hydrolysis was done assuming that the extractives content of CCS samples were solubilized during the acid pretreatment [5]. The liquors generated from the two acid hydrolysis as well as the hydrolysates of the pretreatment were analyzed in a HPLC system equipped with a column 87H (300 × 7.8 mm) Aminex (BioRad) with a RI and UV detectors. This was done to identify the C_6 sugars (i.e., glucose), C_5 sugars (i.e., xylose, arabinose), organic acids (i.e., acetic acid, formic acid) and inhibitors (i.e., HMF and furfural). The oven was adjusted

Table 1
CSF corresponding to the residence time considered in the dilute sulfuric acid pretreatment on CCS.

CSF	1.06	1.36	1.54	1.84	2.01
Residence time (min)	20	40	60	120	180

at 60 °C and the pump was fixed with a flow rate of 0.6 mL/min using as mobile phase a 0.005 M H_2SO_4 solution. Data acquisition and processing were done through the aid of the Varian MS Workstation® software and the regression and graphical analyses with the commercial software (Microsoft Excel by Microsoft, USA). For each component quantified, the coefficient of determination (R^2) was 0.99.

2.4.2. XRD analysis

X-ray diffraction (XRD) is a technique widely used to characterize the degree of crystallinity of lignocellulosic biomass identifying the amount of amorphous cellulose [32]. This analysis was done due to the acid pretreatment changes the cellulose structure through the disruption of the hydrogen bonding of cellulose fibrils [33]. Therefore, the analysis was done to evaluate the rupture of the lignocellulosic matrix of CCS after the dilute acid pretreatment comparing the initial and final degree of crystallinity. Then, the X-ray characterization was carried out using a diffractometer RIGAKU MINIFLEX II with monochromatic Cu-Kα radiation at 30 Kw and 15 mA with a scanning rate of 5°/min (2θ) as well as a sampling width of 0.02°. Scans were obtained in a range from 3° to 50° (2θ) [24]. Therefore, the degree of crystallinity is expressed using the crystallinity index (CrI). This index is the relation between the two forms of the cellulose (i.e., crystalline and amorphous). Finally, the CrI was calculated applying the mathematical expression reported by Segal et al. [25], which is showed below:

$$\%CrI = \frac{I_{002} - I_{am}}{I_{002}} \text{ x } 100 \quad (4)$$

where I_{002} is the highest intensity of the diffraction at $2\theta \approx 22.6°$ and I_{am} is the minimum intensity of the diffraction at $2\theta \approx 19°$. The data acquisition of these intensities was done with the software packages of Origin 8.6®.

2.5. Calculations

The results of the pretreatment step were analyzed calculating different metrics. Indeed, the physicochemical characteristics of the liquid and solid fractions obtained in both acid pretreatment and saccharification were considered. Therefore, equation (5)–10 were used to identify the effect of the pretreatment processes on the CCS. In equations (7) and (8), the terms $Xylose_{CCS}$ and $Arabinose_{CCS}$ correspond to the sugar concentration in the pretreatment step. The values are 23.9 g/L and 1.5 g/L, respectively. The Microsoft Excel software was used to perform these calculations and those corresponding to the statistical analysis.

Component recovery in the treated CCS:

$$\%\text{ Recovery} = \frac{\text{Component in treated CCS x SY}}{\text{Component in raw material}} \text{ x } 100 \quad (5)$$

Component: Cellulose, Hemicellulose, and Lignin.

Component solubilization:

$$\%\text{ Solubilization} = 100 - \%\text{Recovery} \quad (6)$$

Xylose yield:

$$\%\text{Xylose yield} = \frac{C_{Xylose}}{1.13\ Xylose_{CCS}} \text{x } 100 \quad (7)$$

Total sugars released (TSR):

$$\%TSR = \frac{C_{Xylose} + C_{Arabinose}}{1.13(\ Xylose_{CCS} + Arabinose_{CCS})} \times 100 \quad (8)$$

$MW_{xylose}/MW_{xylan} = 1.13$.

Glucose consumed in the synthesis of HMF:

$$GHMF = 1.43\ C_{HMF} \quad (9)$$

$MW_{glucose}/MW_{HMF} = 1.42$.

Xylose consumed in the synthesis of Furfural:

$$XF = 1.56\ C_{Furfual} \quad (10)$$

$MW_{xylose}/MW_{furfural} = 1.56$ (Eq. (10)).

3. Results and discussion

3.1. Effect of the dilute sulfuric acid pretreatment on CCS

The CCS composition in dry basis was (%w/w, db): Extractives (2.55 ± 0.07), glucan (41.53 ± 1.82), xylan (20.64 ± 0.95), arabinan (1.25 ± 0.12), acetyl groups (4.48 ± 0.05), acid insoluble lignin (29.05 ± 0.22) and ash (0.72 ± 0.06). The dilute sulfuric acid pretreatment was analyzed considering the results obtained from the chemical characterization of the solid fraction and the soluble compounds quantification in the liquid fraction produced after the pretreatment. These fractions (i.e., solids and hydrolysates) were analyzed to complete the mass balances of the overall process.

3.1.1. Sugars and inhibitory compounds in hydrolysates

Dilute acid pretreatment has been used to release monomeric sugars able to be considered as a substrate in biotechnological processes to produce ethanol, xylitol, PHB and so forth [26]. Therefore, the identification and analysis of most of the components present in the hydrolysates (i.e., liquid fraction) in terms of sugar and inhibitory compounds concentration must be done. Thus, the concentration of glucose, xylose, and arabinose and sugars yield are showed in Table 2.

Low glucose concentrations were achieved in the hydrolysates (i.e., 0.52 g/L − 1.22 g/L), which indicates a low degradation of the CCS cellulose fraction. This is a typical behavior in this chemical pretreatment. In fact, the hemicellulose fraction is the target of this pretreatment, which explains the high xylose concentrations achieved in all assays (i.e., 8.35 g/L − 15.87 g/L) [27]. The hemicellulose structure composed by xylan, arabinan, mannan, and galactan is hydrolyzed due to the action of the acid catalyst and temperature, which break the chemical bonds of the hemicellulose components. The glucose concentration has an increasing trend respect to the CSF. Indeed, the glucose concentrations starts in 0.52 ± 0.02 g/L and finish in 1.22 ± 0.02 g/L. Then, the severities used represent a low effect on the cellulose hydrolysis since the glucose yields have values lower of 3%. In contrast, the arabinose concentration was reduced until a value of 0.63 ± 0.07 g/L with the increase of the severity. Thus, the arabinose yields were higher than 60% at low severity conditions and suddenly reduced until a value of 42.85% for the CSF of 2.01. Nevertheless, the arabinose released did not affect the TSR in comparison with the xylose. Sugar yields decrease at CSF of 2.01 (1.62%, 36.02% and 42.85% for glucose, xylose and arabinose, respectively), since the increase of residence time allows the degradation of sugars to other compounds, such as acetic acid, formic acid, HMF, furfural, among others. The maximum xylose concentration and yield were reached at CSF of 1.84. The values were 15.87 ± 0.13 g/L and 65.95%, respectively. The concentration disagreed with the report of Aristizábal et al. [17], for the CCS. The xylose concentration of 19.58 g/L was found at 115 °C and 30 min of

residence time (the CSF calculated corresponds to 1.08). Nevertheless, the xylose yield in this research represents 50% of the potential of the CCS. In this sense, the increase of the pretreatment severity allows obtaining high efficiencies related to the xylan degradation. Therefore, the best condition to obtain a high TRS was applying a CSF equals to 1.84 with a value of 66.75%. This value is similar to the hemicellulose recovery reported by Martinez et al. [28], using olive tree biomass as raw material and acid pretreatment conditions of 160 °C, 25 and 35 %w/w (i.e., solids loading), 10 min of residence time, and acid concentrations of 4 and 8 %w/w.

The CCS xylose yields are lower than those obtained from the acid pretreatment of sugarcane bagasse and rice husk [17]. The obtained allows identifying the diverse effect of the pretreatment variables (i.e. residence time and acid concentration) with a fixed temperature of 120 °C and solid: liquid ratio of 1:10 on the xylose yield. In fact, the obtained results are comparable with the xylose yields reported for hardwoods and softwoods [29]. In this sense, high xylose yields are obtained increasing both residence time and acid concentration in hardwood raw materials. This statement is corroborated by Parajo et al. [9], which needs a 3.5% sulfuric acid solution and 10 h of residence time to perform the acid pretreatment of Eucalyptus. Thus, the pretreatment severity differs strongly when agricultural residues and woody biomass are used as raw materials. In fact, CCS gives a xylose yield of 34.68% applying a CSF of 1.07, while xylose yields of 75.9% and 75.6 for rice straw and rice husk are reported, respectively [30]. Hence, the research of the best acid pretreatment conditions for woody biomass is should be done avoiding the use of the typical values reported in the literature. Moreover, high xylose yields could be achieved high temperatures and low residence times, solid: liquid ratio, and acid concentrations. However, the thermal degradation of the sugars is a risk factor. For instance, Canettieri et al. [31], reports an optimal point to pretreat Eucalyptus using a solid: liquid ratio of 1:8.6, sulfuric acid concentration of 0.65 %w/w, 157 °C, and a residence time (20 min) to get a xylose yield of 79.6%.

Table 3 presents the concentrations of other compounds (i.e., furaldehydes and aliphatic acids) found during the compositional analysis of the hydrolysates. They are derived from the hemicellulose deacetylation and the dehydration of sugars during the pretreatment. The presence of inhibitor compounds decrease the sugars consumption of different microorganisms, which reduces the fermentation productivity [32]. Among the aliphatic acids detected, significant quantities of acetic acid were liberated from the acetyl groups contained in the CCS until be fully hydrolyzed. A same behavior as for the sugars was presented for the acetic acid concentration increasing with the CSF until a concentration of 4.79 ± 0.05 g/L due to a large amount of acetyl groups in CCS. After the CSF of 1.84, the concentration was reduced until the half. The increase in the concentration of the inhibitory compounds (such as acetic acid, formic acid, HMF and furfural) is a product of the increase in residence time. Hemicellulose is mainly composed of the sugars xylose and arabinose (i.e., pentoses) and glucose, galactose and mannose (i.e., hexoses) in different proportions [33]. However, when degradation of these sugars occurs, the release of inhibitory compounds is triggered. In the case of xylose, degradation generates the release of furfural, and of arabinose and glucose, components such as formic acid and acetic acid are produced [34]. Therefore, an increase in the concentrations of furfural and acetic acid at CSF of 2.01 is evidenced. The maximum acetic acid concentration found is low in comparison with the concentrations reported in the literature (i.e., 7 or 10 g/L) [35]. Even so, the produced hydrolysates cannot be used directly in a fermentation process due to the possible presence of inhibitory compounds. In this way, the analysis of the suitability of the hydrolysates for fermentation must be carried out in a more specific and rigorous way. Indeed, different factors can affect a fermentation process to produce different pentose-based products through biotechnological processes (e.g., xylitol) [36]. Nevertheless, a detoxification stage should be performed to avoid the presence of inhibitory compounds in the fermentation media. Moreover, the presence of dissolved inorganic compounds and toxic components should be tested and avoided through a detoxification stage. Finally, Moreover, the pretreatment process does not have a high lignin solubilization due to the adverse effects of phenolic compound in fermentation processes [37].

The furaldehydes such as HMF and furfural were produced in most of the evaluated severity conditions due to the dehydration of glucose and xylose, while the formic acid is present by the hydration of HMF [38]. The concentration of these can be negligible compared with other reports since just values above 1 g/L were achieved. This concentration affects the cell growth and productivity of microorganism [39]. Nevertheless, the concentrations of HMF and formic acid at the two final conditions (i.e., CSF = 1.84 and CSF = 2.01) were not detected by liquid chromatography. Then, a glucose concentration of 0.52–0.66 g/L was degraded until HMF and a xylose concentration of 0.079–0.702 g/L was converted in furfural during the pretreatment. The furfural concentrations quantified by HPLC analysis of the CCS hydrolysates are lower than the reported by different authors using similar pretreatment conditions in terms of residence time and temperature. For instance, Yu et al. [40], report a furfural concentration of 5 g/L using sulfuric acid at 0.01 %w/w after to pretreat cassava. Moreover, the furfural concentrations are lower than the data reported by Chiranjeevi et al. [41], (i.e., 2.40 g/L) using sulfuric acid at 0.75 %w/w after pretreating rice straw. In this way, the low furfural concentrations obtained in the CCS hydrolysates are attributed to the low xylose yield and conversion obtained, which were explained due to the rigid structure of woody biomass. Thus, the low furfural concentrations are a consequence of both the acid pretreatment operating conditions and the type of biomass.

3.1.2. Solid fraction analysis

The dry matter content of the treated solids was 34 ± 1.7%, which corresponds to a SY between 0.68 ± 0.01 and 0.85 ± 0.04 for

Table 2
Sugar concentration and yields of the hydrolysate derived from the dilute sulfuric acid pretreatment of CCS.

Component	CSF														
	1.06			1.36			1.54			1.84			2.01		
Glucose (g/L)	0.52	±	0.02	0.72	±	0.01	0.91	±	0.02	1.22	±	0.02	0.78	±	0.06
Xylose (g/L)	8.35	±	0.11	11.36	±	0.01	12.91	±	0.18	15.87	±	0.13	8.67	±	0.87
Arabinose (g/L)	1.00	±	0.04	0.94	±	0.01	0.96	±	0.01	1.07	±	0.02	0.63	±	0.07
Glucose yield (%)	1.08	±	0.08	1.49	±	0.06	1.88	±	0.09	2.52	±	0.08	1.62	±	0.10
Xylose yield	34.68	±	0.06	47.19	±	0.02	53.64	±	0.08	65.95	±	0.12	36.02	±	1.01
Arabinose yield (%)	68.62	±	0.14	64.24	±	0.02	65.50	±	0.02	73.42	±	0.04	42.85	±	0.09
TSR (%)	36.83	±	0.19	48.44	±	0.09	54.63	±	0.17	66.75	±	0.11	36.62	±	1.21

the CSF of 2.01 and 1.06, respectively. Then, the amount of solids recovered in the acid pretreatment process decreases with the severity [42]. This can expressed using the linear correlation % SY = −18.10 CSF +103.5 (R^2 = 0.98). In addition, the obtained SY results are in concordance with the wood yield reported by Gütsch et al. [43], applying a high residence time, low acid concentration, and temperature in Eucalyptus samples.

In Fig. 1, the results of the chemical composition of the treated CCS are presented. The cellulose fraction was analyzed in terms of glucan, while the hemicellulose fraction was analyzed in terms of xylan, arabinan, and acetyl groups. The same behavior was observed for each component of the treated solid respect to the CSF. Although no direct relation between the CSF or the SY and the glucan, lignin, arabinan, and acetyl groups content was found. In fact, a linear relation was presented for the CSF and the xylan content and solubilization with an R^2 = 0.92. The equation for the xylan content was $Xylan_{CCS}$ = −2.02 CSF +12.434.

The glucan and lignin content were reduced with the increase of the severity factor obtaining high recoveries in the solid fraction (i.e., glucan: 63.0%–105% and lignin: 88.40%–90.69%). Eventually, it was expected a reduction in all the cases for the glucan content since in the hydrolysates a consecutive increase in the glucose content was observed. However, the CSF of 1.36 allows having a high content of 45 ± 2.6% with a recovery of 104.56%. However, a decrease in the glucan content is perceived when the severity factor increases from 1.36. This is related to its solubilization in the medium, due to the high residence times that favor this process. In addition, a low lignin content of 26.2 ± 0.4% was observed corresponding to a high percentage of lignin solubilization of 12.2%. Therefore, this CSF can have a high effect on the disruption of the cross-linked matrix of the lignin and hemicellulose, thus the cellulose content embedded by the hemicellulose fraction could be able to be detected in the treated CCS. This behavior was also found by Gütsch et al. [43], for Eucalyptus treated at 120 °C, 36 min and a sulfuric 0.1 M. The glucan content in the base at raw material was 40.33% compared with the 39.9% and the highest lignin solubilization of 24% for the same temperature. In this way, low temperature and residence time increase the glucan content respect to the raw material. Nevertheless, another technique to study the chemical changes of lignin and carbohydrates has to be applied to corroborate this behavior [44]. On the other hand, the reduction of the lignin content in all cases shows a lignin re-condensation during the dilute acid pretreatment was not present as in the case of the olive tree biomass considered by Martínez-Patiño et al., [28].

The hemicellulose fraction was the main lignocellulosic compound removed in all assays. The arabinan and acetyl groups were removed totality in all cases. The acetyl groups content in the other cases remains linked to the xylan backbone in a range of 1.7 ± 0.1% for the CSF of 1.54 and almost the half of the present in the raw material at CSF of 1.06 (i.e. 2.4 ± 0.5%). Thus, high CSF values allow a complete hemicellulose deacetylation. The xylan content was 9.7 ± 2.0%, 9.5 ± 0.6%, 6.9 ± 0.2%, 3.2 ± 0.1% and 2.7 ± 0.8% for each CSF 1.06, 1.36, 1.54, 1.84 and 2.01, respectively. These results denote a great solubilization of the xylan, with the high value of 87.19% for the CSF of 2.01 and 100% for the arabinan and acetyl groups. Even though, the CSF of 2.01 represents the maximal point of hemicellulose fraction removal for the solid fraction, while in the liquid fraction the maximal point of TSR was the 1.86 due to the degradation of the sugars. Therefore, for the conditions of pretreatment selected, the increase of the residence time reduces the sugar release of the hemicellulose present in the CCS and an optimal point would be between the CSF of 1.54 and 2.01. Additionally, an increase of the temperature reduces the residence time and the acid concentration could represent a better scenario to have low glucan solubilization compared with the hemicellulose solubilization of 91.7% reported for Poplar by Kumar et al., [45]. Nevertheless, the increase of temperature to 190 °C can represent an increase in the energy demand of this process step, which should be studied at the same time in terms of techno-economic assessments [46].

3.2. Enzymatic digestibility (ED)

The research seeks to improve the ED of CCS using the dilute sulfuric acid pretreatment to overcome the recalcitrance of this feedstock. This objective was formulated in the wake of the low glucose concentration obtained during the direct saccharification of CCS (i.e., 1 g/L). The results obtained for the saccharification process are presented in Fig. 2. An increase in the glucose concentration over the time was a general trend observed during the saccharification step of the treated solids. In fact, a notorious increase in the glucose release with the increase of the CSF was present. Therefore, the CSF of 2.01 achieves the maximal glucose release of 9.53 ± 0.01 g/L at 72 h compared with the 2.74 g/L at CSF of 1.06. Thus, the ED corresponds to 43.40% and 12.01%, respectively. Then, the cellulose conversion of 19.67% achieved by Aristizábal et al. [17], with Celluclast 1.5 L and viscozyme was improved. In addition, the CSF of 2.01 has a great effect on the rate of glucose generated since at 7 h of saccharification more than 50% of the final glucose concentration was reached.

Nevertheless, a complete cellulose conversion was not achieved in the last CSF (i.e., 2.01). In this sense, a β-glucosidase supplementation was performed in order to show an improvement in the digestibility. The main action of this enzyme is to hydrolyze cellobiose to glucose. The glucose concentration was increased 74% respect to the assay without β-glucosidase supplementation at 48 h. At this time, the concentration was similar to the reported value by Martínez et al. [47], in the olive tree pruning treated with phosphoric acid in a concentration of 1.5% at 170 °C during 10 min and a 30% total solids loading. Moreover, the total operation was extended to 117 h but only a difference of 2 g/L was observed after 48 h. Therefore, the ED value was 64.13% representing an increase of 59% respect to the data without β-glucosidase supplementation.

In terms of the total glucose yield, a linear correlation was found with the severity. The highest value was 64.13% with the solids treated with a CSF of 2.01 and β-glucosidase supplementation. Even

Table 3
Concentration of Inhibitory compounds in the liquid fraction from the dilute sulfuric acid pretreatment of CCS.

Component	CSF													
	1.06			1.36			1.54			1.84			2.01		
Acetic acid	3.18	±	0.01	3.55	±	0.11	3.78	±	0.03	4.79	±	0.05	5.20	±	0.18
Formic acid	0.11	±	0.01	0.24	±	0.04	0.64	±	0.01	N.D.			N.D.		
HMF	0.36	±	0.04	0.38	±	0.10	0.47	±	0.11	N.D.			N.D.		
Furfural	0.05	±	0.01	0.09	±	0.02	0.14	±	0.01	0.58	±	0.07	0.61	±	0.05

N.D.: Not detected.

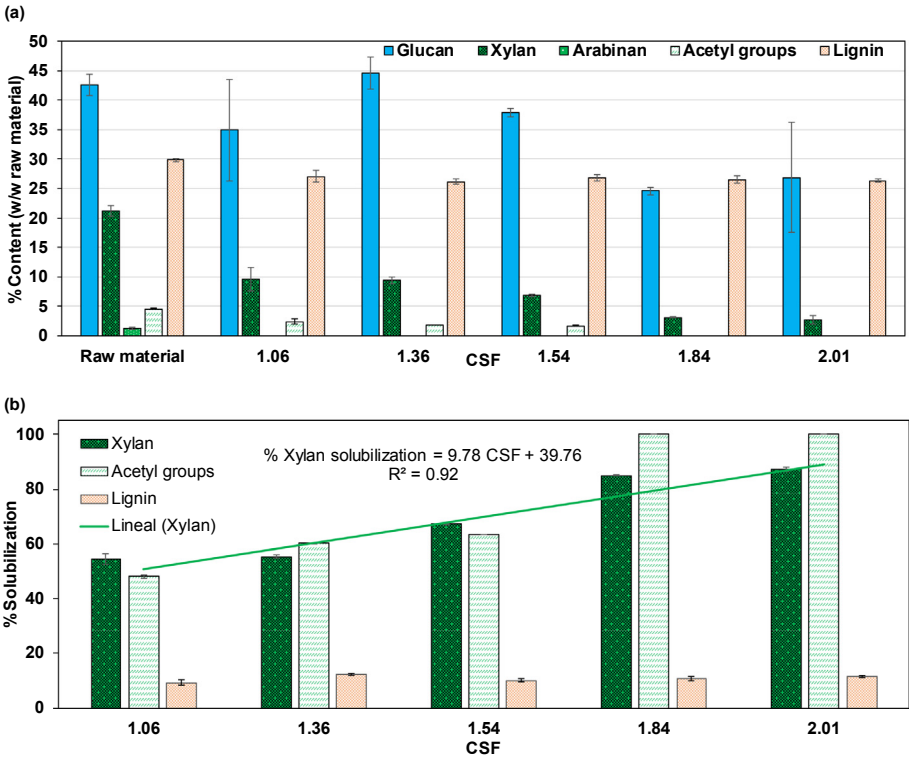

Fig. 1. Effect of the CSF in the treated solids derived of the dilute sulfuric acid pretreatment of CCS. **a.** Lignocellulosic components. **b.** Solubilization percentage of the lignocellulosic components.

though, this supplementation did not complete cellulose conversion of the raw material. In this case, the CSF of 2.01 allows the high total glucose yield, while the maximum value of the TSR is reached using a CSF of 1.84. Thus, two global mass balances can be performed depending on the purpose of the pretreatment process.

3.3. Crystallinity index of the raw material and pretreated solids

The disruption of the lignocellulose structure of CCS with and without the pretreatment was analyzed through the CrI. The results are presented in Fig. 3. These results were associated with the CSF applying a nonlinear correlation with an $R^2 = 0.91$. For each assay, an increase in the crystallinity index respect to the CCS was identified. However, a value of 54% was detected when the CSF = 1.06. Therefore, the increase respect to the feedstock corresponds to 33%, 29%, and 23%, respectively. Moreover, the increase of the CrI is possible owing to the removal of amorphous regions of cellulose, hemicellulose and lignin [48]. This result is similar to the data obtained for other hardwoods. In fact, Carrasco et al. [49], has applied conditions of 120 °C, 1 (%v/v H_2SO_4), a solid liquid ration of 1: 10, and different residence times on different hardwoods such as Poplar, Oak wood, and Eucalyptus. For each case, an increasing tendency of 15%, 17.54%, and 28.14% was found. While, Kumar et al. [46], only achieve an increase in Poplar of 1.4% handling a temperature of 190 °C, the residence time of 70 s and a low concentration of sulfuric acid of 0.6% and 4.5% [45]. The same happens with the investigation of Carvalho et al. [52], where an increase of 19.52% for Eucalyptus with a high temperature of 175 °C, low residence time 15 min and concentration of sulfuric acid of 4.5% is observed. Then, these comparisons lead to identify two important factors of this index. The first factor is related to the increase of the CrI at low temperatures and acid concentrations using hardwoods as raw materials. The other statement is related to CCS could have a structure more amorphous than other hardwoods researched in this field since they have a high CrI. Furthermore, a linear correlation with an $R^2 = 0.95$ between the %CrI and the total glucose yield was found. The equation was %Total glucose yield = 8.38 %CrI − 5.24.

3.4. Mass balance

Two mass balances were constructed using the data obtained in the acid pretreatment and subsequent enzymatic hydrolysis of CCS. These are presented in Fig. 4. In both cases, 100 g of dry CCS and 1000 g of sulfuric acid solution were fed to the pretreatment. The CCS contain 42.62 g, 21.18 g, 1.29 g, 4.59 g, and 29.81 g of glucan, xylan, arabinan, acetyl groups, and lignin, respectively. In the pretreatment stage, the residence time is the difference between the two scheme (i.e., 120 min and 180 min). In each scheme, 70 g and 68 g of pretreated CCS are generated. Moreover, 771.3 g and 730 g of hydrolysates are produced. In the first scheme 0.82 g of arabinose and 12.24 g of xylose were released. In the second scheme the amounts of arabinose and xylose were reduced 50%. The same happens with the glucan content corresponding to recoveries of 57.77% and 63% in schemes **a** and **b**, respectively. This content is submitted to an enzymatic hydrolysis releasing 10.75 and 20.63 g of glucose. The possibilities to use these quantities in ethanol production are better for the scheme **b** since considering a synthesis of 90% of the theoretical yield of stoichiometric reaction around of 12.02 ml per 100 g of CCS can be produced. Furthermore, 7 ml of butanol per 100 g of CCS can be produced assuming the yield of 0.27 g butanol/g glucose reported by Al-Shorgani et al. [50], and Jaramillo et al., [51]. Moreover, the scheme **a** can be used for xylitol production with a rate of 8.93 g per 100 g of CCS considering the yield of 0.73 g/g reported by Roberto et al. [52], using *Candida guilliermondii* as microorganism. Nevertheless, concentration,

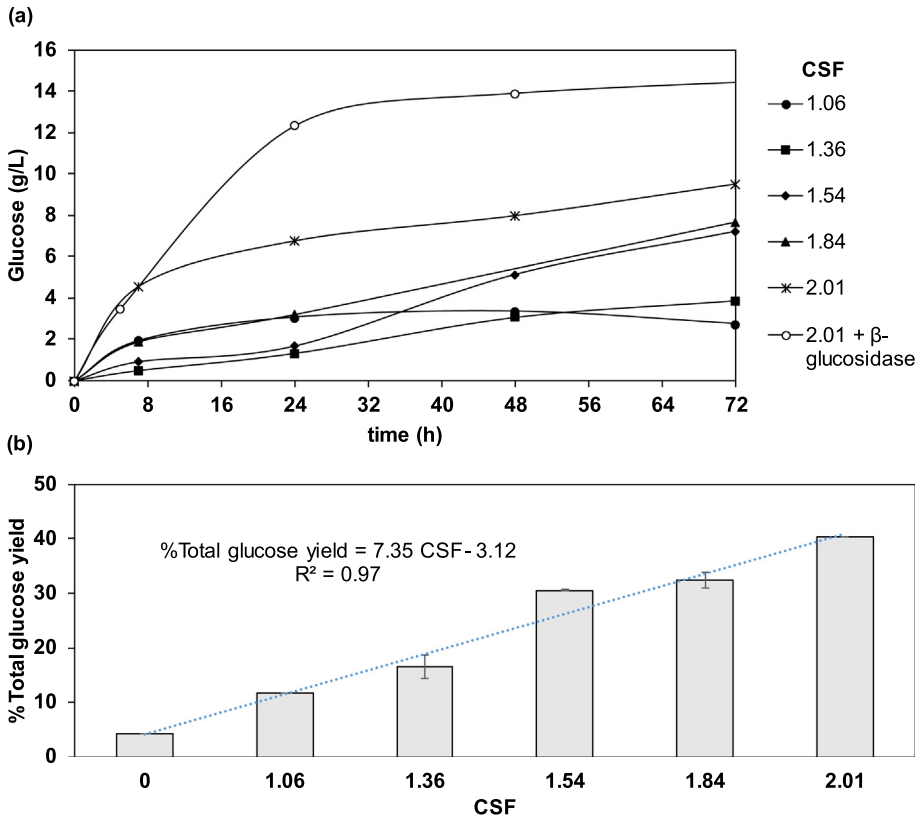

Fig. 2. a. Profile of glucose concentration of enzymatic hydrolysis of the CCS treated with dilute acid pretreatment. **b.** Correlation between CSF and total glucose yield.

detoxification and purification of both hydrolysates (i.e., rich in pentoses or hexoses) should be done. These additional steps can increase the overall cost of the process.

The mass balances presented can be used to determine the performance of the process in terms of mass and energy indicators. Several authors have reported these indicators. In this way, Ruiz-Mercado et al. [53], summarizes more than 26 mass efficiency indicators and 15 energy indicators. Nevertheless, only the process mass intensity (PMI) index was calculated. This index involves all the input streams and the desired product. Thus, the PMI index is calculated as the ratio between all the input streams and the mass flow of the desired product (i.e., liquid stream from the saccharification process) [54]. The PMI of both schemes is 1.92 and 2.03, respectively. These values are in agreement with the reported PMI values for the chemical industry, which varies from 5 to 50 [55]. However, other processing stages are missing (e.g., fermentation), which can increase the value of the indicator. On the other hand, the input and output energy balance of the process avoiding the utilities requirements can be done. The energy content of 1 kg of CCS is about 19.32 MJ, while the energy content of 1 kg of glucose is 15.55 MJ. In this way, the output to input energy ratio of the pretreatment process is 8.32% and 16.61% in the first and second

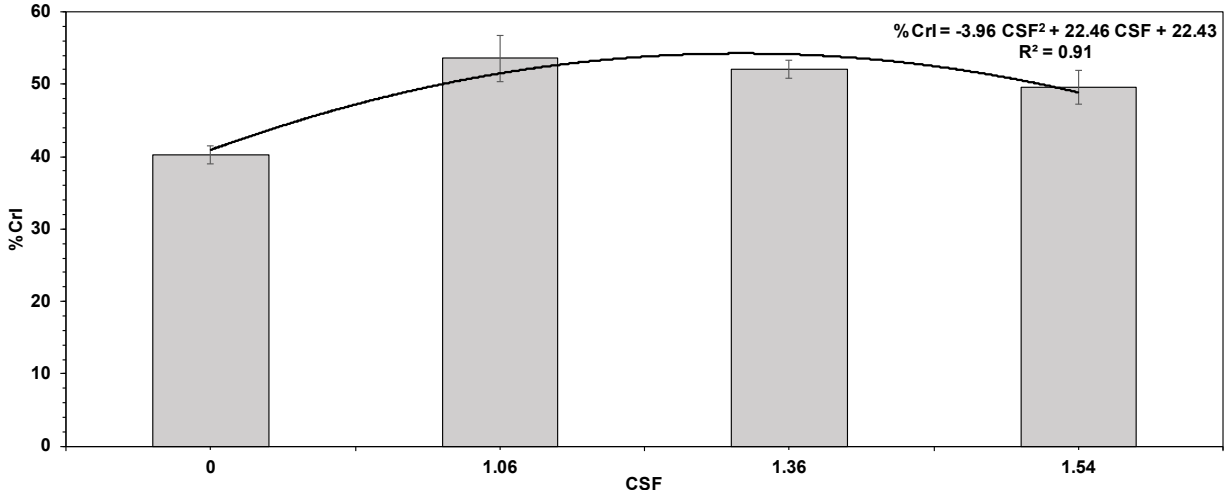

Fig. 3. CrI of CCS and treated solids derived of the dilute sulfuric acid pretreatment.

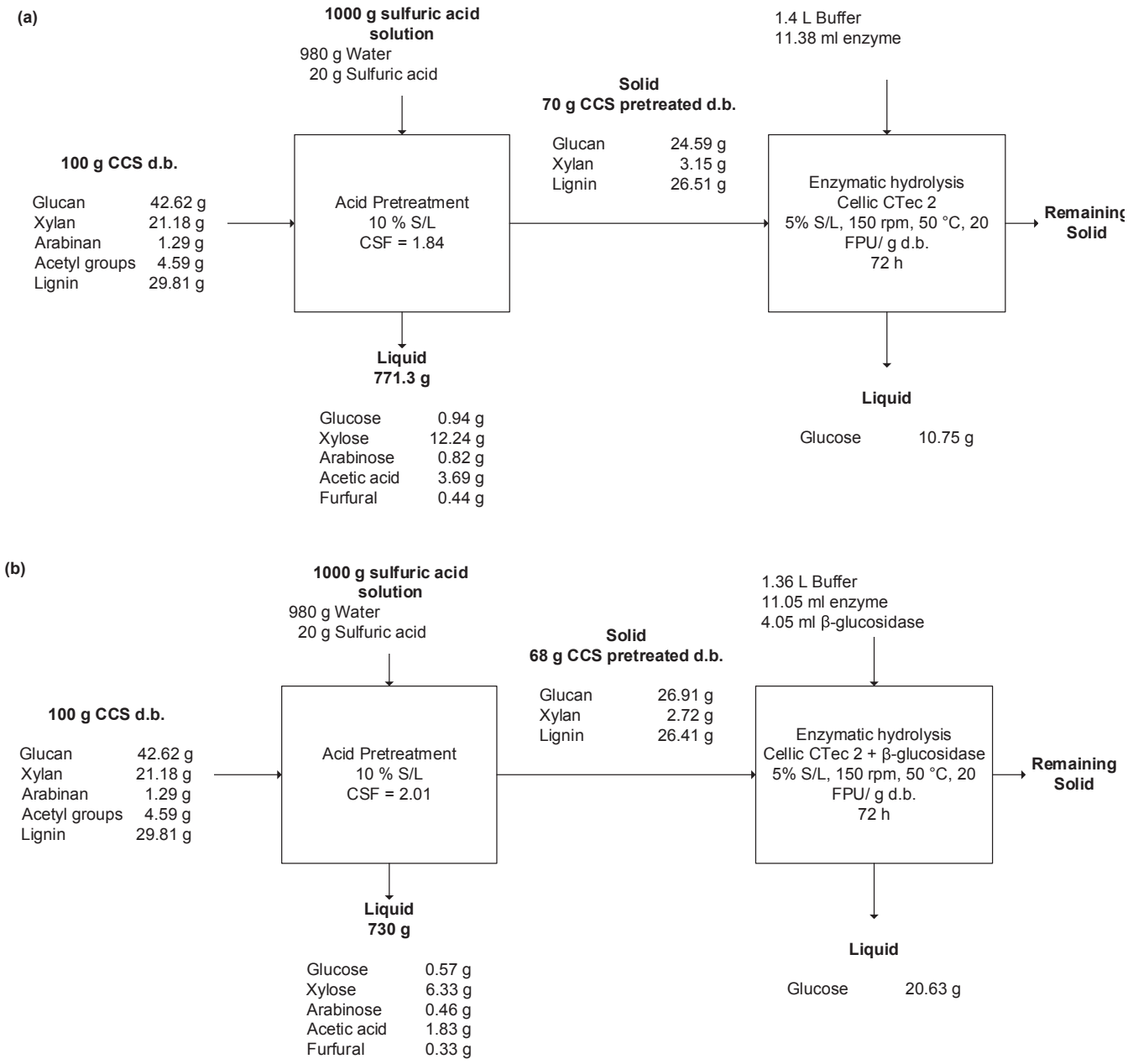

Fig. 4. Mass balance flow scheme of the overall process for the sugar release from CCS after dilute acid pretreatment. **a**. CSF of 1.08 and **b.** CSF of 2.01.

schemes, respectively. These values suggest high-energy losses in both acid pretreatment and saccharification process due to the chemical conversion of the lignocellulosic matrix of the raw material. Therefore, the pretreatment stage of lignocellulosic biomass could be considered as one of the most inefficient processes in a biomass upgrading when using biotechnological conversion routes as main processing lines. This statement is in agreement with the simulation results reported by Piarpuzán et al. [56], where the chemical pretreatment process and saccharification stages consume more than 20% of the total energy input to the bioethanol production process.

The mass balances of the process can be used as input data to perform a simulation of the dilute acid pretreatment and saccharification process of CCS. From this, an economic analysis can be done. Nevertheless, a simulation of the process should be carried out to size the equipment in the process as well as find the total equipment cost. The conditions discussed to enhance the glucose yield of CCS allows suggesting low costs associated to maintenance and depreciation due to the use of low temperatures and acid concentrations. Finally, the use of sulfuric acid as catalyst to disrupt the lignocellulosic matrix of CCS can decrease the sustainability of the process. In fact, the use of sulfuric acid reduces the environmental sustainability of the process due to the possible releases of toxic compounds, which can affect environmental impact categories such as aquatic toxicity potential, terrestrial toxicity potential, an acidification potential. On the other hand, the social impact of the process is affected due to the use of hazardous materials, which can affect the health of the workers [58]. For these reasons, the overall sustainability of the acid pretreatment process should be improved using heterogeneous catalysts such as acid zeolites, which can reduce the environmental and social impact of the process as well as decrease the operational expenditures of the

entire pretreatment stage [26].

4. Conclusions

The acid pretreatment and saccharification process were carried out to produce fermentable sugars using coffee-cut stems as raw materials. In the acid pretreatment process, two optimal pretreatment conditions were obtained depending on the purpose of the sugars production process. The first optimal conditions involve to pretreat the coffee-cut stems at 120 °C, 180 min, and acid concentration of 2%w/w (i.e., CSF 2.01) in the dilute acid process. Then, add β −glucosidase in the saccharification process to obtain a high glucose yield. Moreover, the addition of β −glucosidase reduces the residence time of the saccharification process allowing to reach a maximum cellulose conversion in 24 h. The second optimal conditions involve to pretreat the coffee-cut stems at 120 °C, 120 min, and acid concentration of 2%w/w (i.e., CSF 1.84) in the dilute acid process. These conditions allow obtaining maximum total sugars recovery in the pre-hydrolysate liquor. Therefore, another biotechnological processes related to the xylose conversion can be included in the overall valorization of this residue. Finally, the mass balances of the process as well as the optimal conditions of the acid pretreatment process are the basis to simulate the real potential of coffee cut-stems as alternative feedstock to produce sugar-based products such as bioethanol, biobutanol, biogas, lactic acid, and xylitol avoiding the use of unselective kinetic models.

Declaration of competing interest

None.

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